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TITLE: Development of Antibodies Against Novel Cell Surface Proteins in Hormone Refractory Prostate Cancer

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14. ABSTRACT N-cadherin is a cell surface marker that is overexpressed in hormone refractory prostate cancer and targeting this protein either diagnostically or therapeutically may have clinical utility. We initially observed that the cadherin profiles of certain prostate cancer cell lines and xenografts correlated with their level of invasiveness. In addition, we saw that amongst certain hormone refractory xenograft models, there was a consistent upregulation of N-cadherin when compared to its androgen dependent counterpart. We generated specific monoclonal antibodies against different domains of N-cadherin in an attempt to determine if blockade could decrease invasion and metastasis. In the first two years of the grant, we characterized these antibodies and determined that they had measurable in vitro activity. For the third year of the grant, there has been an increased focus on the in vivo effects of two specific antibodies that were generated and their mechanism of action.					
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INTRODUCTION

The process known as Epithelial-Mesenchymal Transition (EMT) continues to be implicated as a crucial mediator of carcinogenesis. This occurrence is manifested by “Cadherin Switching” in which Epithelial (E) cadherin is lost and Neuronal (N) cadherin is upregulated. We observed that the cadherin profiles of certain prostate cancer cell lines and xenografts correlated with their level of invasiveness. Evidence suggested that blocking the switch from E-cadherin to N-cadherin may block motility and invasiveness of prostate cells *in vitro*. We sought to validate N-cadherin as a target for diagnostics or therapeutic purposes in hormone refractory prostate cancer and determine if blockade of this pathway could decrease invasion and metastasis. Previous studies have demonstrated that certain domains of the protein have unique activity and contribute in different ways to the various functions of N-cadherin. As a result, we generated two specific monoclonal antibodies against N-cadherin; one against the first extracellular domain and one against the 4th extracellular domain.

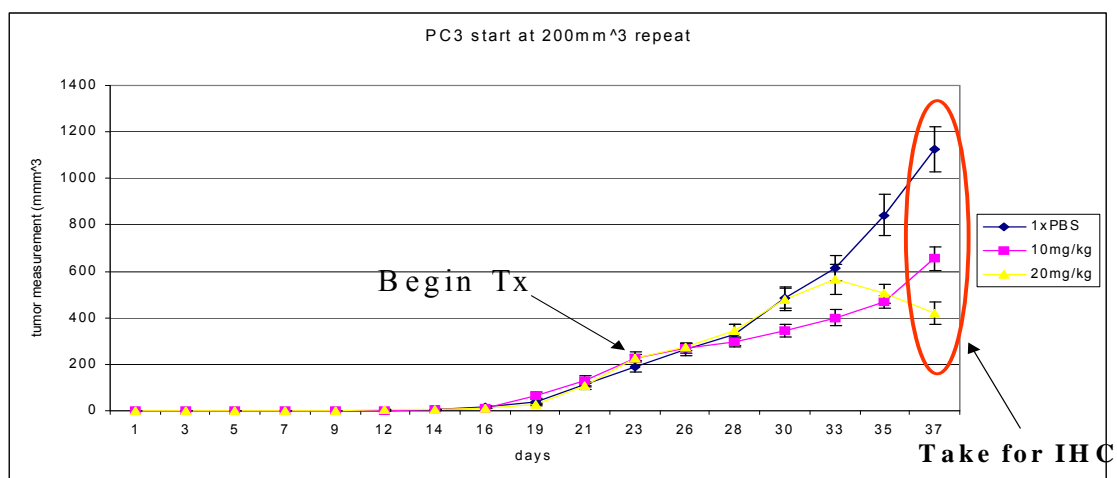
PROGRESS REPORT

Specific Aim 3. Preclinical testing

Subaim 3.1 Intact fully human antibodies for *in vitro* and *in vivo* pre-clinical testing

To study the involvement of N-cadherin, we looked at the effect of our antibodies against N-cadherin (EC4) on tumor growth. PC3 cells were injected into castrated mice and treated with N-cadherin antibody when tumors became palpable. There was a clear tumor growth inhibition and with 20mg/kg of antibody, we saw the tumors become immeasurable. With the experiment below, we harvested tumors before the 20mg/kg reached zero. Tumors were harvested for histological and molecular analysis (in progress). We are currently testing for EMT, death, apoptosis, and angiogenesis markers to try and better understand the nature of the tumors.

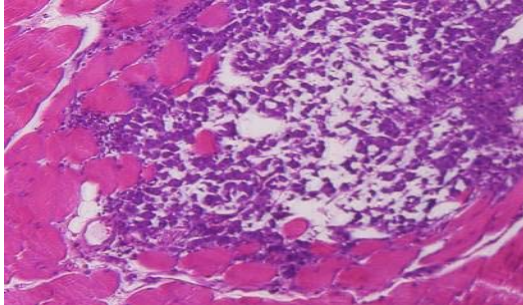
PC3: EC4 treatment at 200mm³ repeat



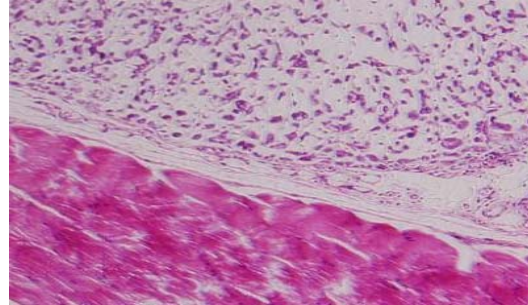
Below, PBS control shows cells invading into the muscle. Anti N-cadherin antibody treated tumors show no invasion into neighboring tissue.

PC3 tumors treated with anti-Ncadherin antibody

PBS control

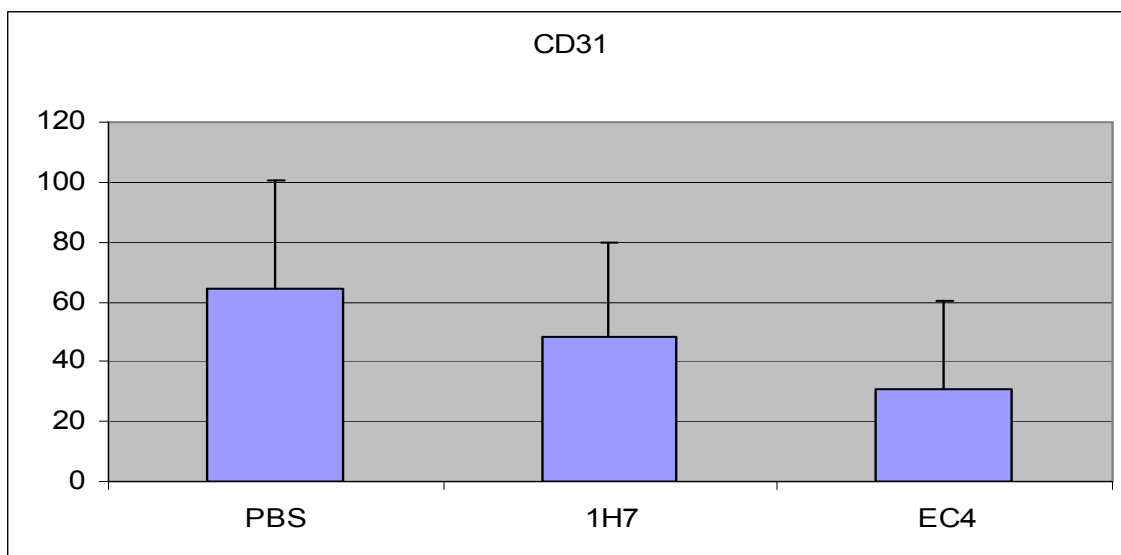


EC 4 (10mg/kg) treated



10x magnification

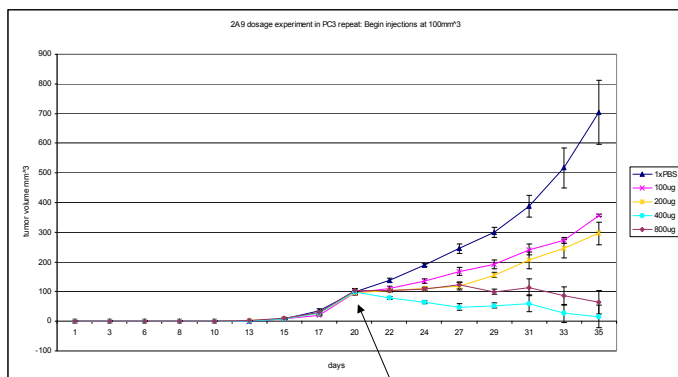
We also have started to address the mechanism of action of the anti N-cadherin antibodies. Preliminary evidence suggests that inhibition of angiogenesis may play a role. Below, immunohistochemical staining of CD31 suggests a decrease in antibody treated animals compared to control.



Subaim 3.3 Combination of antibodies with other therapies

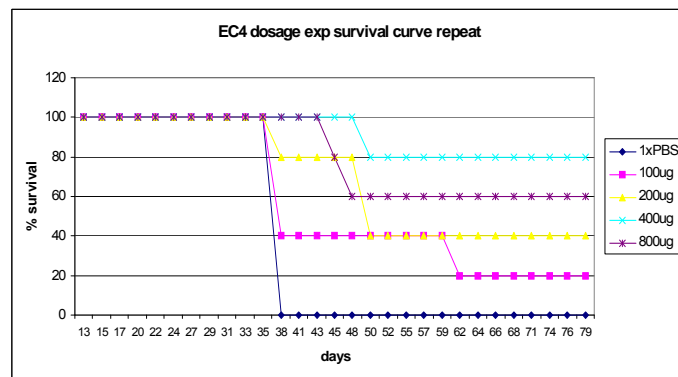
The use of N-cadherin antibodies may be enhanced with other therapies. In order to find an antibody concentration that will allow us to observe additive or synergistic benefits, dose escalation experiments have been completed for the N-cadherin antibody to the 4th Extracellular Domain. Dose escalation experiments are in progress for Taxotere chemotherapy which is widely considered the standard of care for hormone refractory prostate cancer.

PC3: EC4 dose escalation at 100mm³ repeat



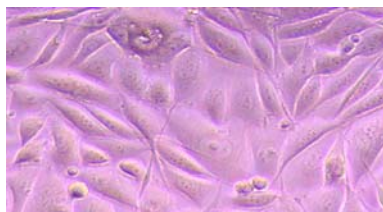
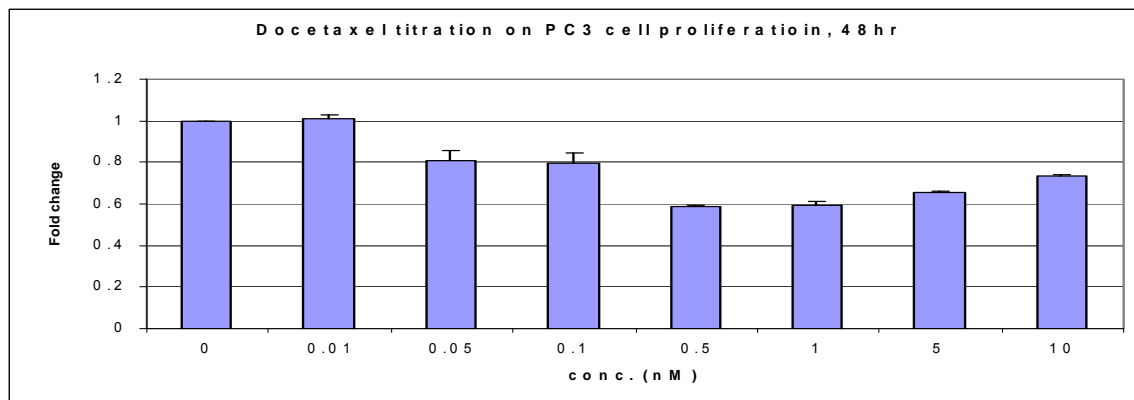
Begin Tx

PC3: EC4 dose escalation at 100mm³ survival

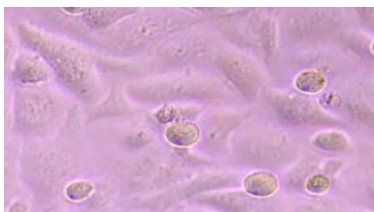


100ug: 1 small tumor
200ug: 2/2 small tumor
400ug: 4/5 no tumor
800ug: 2/3 no tumor, 1/3 small tumor

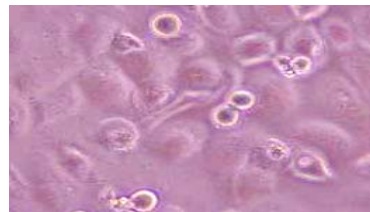
The below figure is an in vitro titration of Taxotere. In vitro and in vivo experiments are currently in progress using combination therapy of Taxotere and anti-N-cadherin antibody.



0nM (control)

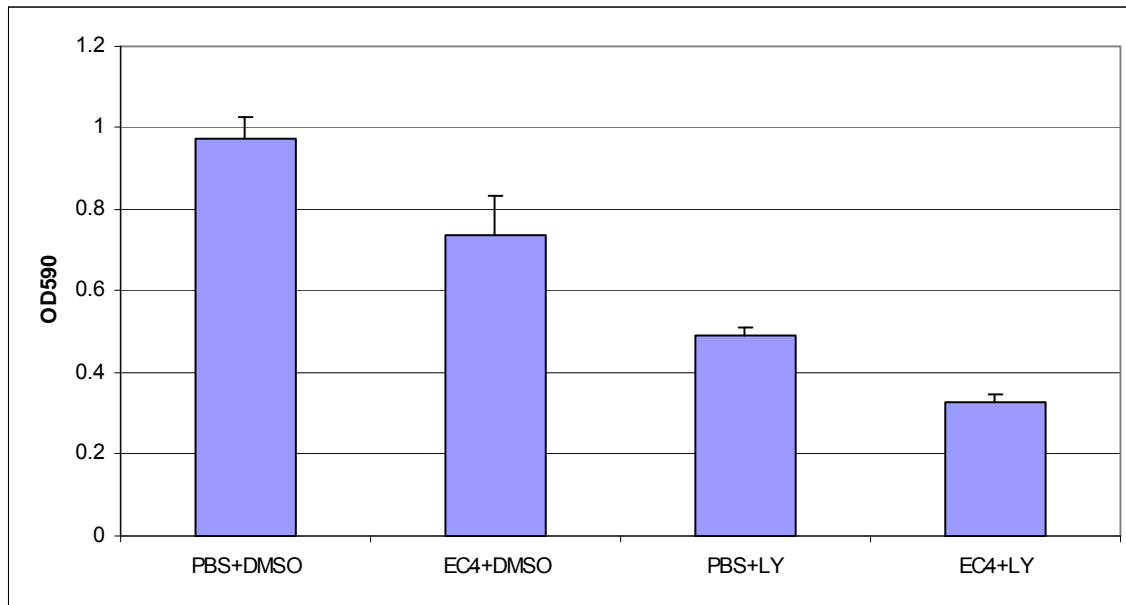


0.1 nM



1.0 nM

Additional in vitro work to look at other possible combination therapies are in progress. In vitro ground work has also been started using LY294002 (an inhibitor of the PI3 Kinase pathway) as additional targeted agents. Below, we demonstrate preliminary evidence that the combination of an anti N-cadherin antibody and a PI3 Kinase inhibitor behave synergistically by inhibiting PC3 invasion.



KEY RESEARCH ACCOMPLISHMENTS

- In vivo N-cadherin treated tumors showed no signs of invasion into neighboring tissue by H&E.
- There are several candidate targets that are showing promise as possible combination therapies with the N-cadherin antibody.
- The mechanism of action of the antibody is being thoroughly investigated and may involve angiogenesis.

REPORTABLE OUTCOMES

None

CONCLUSION

We have demonstrated in several xenograft mouse models that N-cadherin-antibody treatment decreases the size and invasiveness (by H&E) of implanted tumors. We are currently testing for possible markers that could give us a hint to possible mechanisms of action including inhibition of angiogenesis or blockade of other signaling cascades. We are also doing preliminary in vitro and in vivo work on possible target therapies that can be used in combination with our antibodies. We are planning to determine if our antibody affects rates of metastasis in various models. A manuscript is in preparation.

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